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Serum Lipid Profile And Random Blood Glucose of Male And Female Wistar Rats Following Administration of Leptin Hormone After A Dietary Regime

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***Abstract*—The Leptin hormone is a product of the obesity gene, a key regulator of feeding and energy expenditure.The World Health Organization (WHO) predicts 11.1 million deaths globally and 71% deaths in developing countries due to Coronary Artery Disease (CAD) by 2020 A.D. CAD has been associated with alterations in lipid metabolism, which include hyper-triglyceridemia and significantly reduced HDL-c. The increase in the concentration of cholesterol is attributable to LDL cholesterol because the high density lipoprotein (HDL) cholesterol is typically reduced. The study is aimed at determining the effect of leptin hormone on fasting serum lipid profile, atherogenic index (AI) and Random Blood glucose (RBG) inWistar rats following a diet regime and leptin injection.Fourty (40) Wistar rats (male n=20, female=20), age 9weeks and weighing between 77.2g-123.0g were randomly divided into two (2) groups of 5 rats per sub-group. All groups were maintained ad-libitum on experimental diets and water for a period of 10weeks. Group 1 (A_1 and B_1); the control received standard rat feed, group 2 (A_2 and B_2) received high fat diet (margarine, 90%) mixed with some standard rat feed (10%), group 3 (A_3 and B_3) received protein diet (soya beans, 100%) and group 4 (A_4 and B_4) received carbohydrate diet (cereal, 100%). The leptin hormone was given intra-peritoneal for a period of two (2) weeks.The resultshowed a significant difference ($P<0.05$) in the Tot-c, TG, VLDL-c, LDL-c and RBG in the pre-test and post-test periods, but there was no significant difference ($P>0.05$) for the calculated atherogenic index (AI). There was a significant difference ($P<0.05$) and sexual dimorphism for HDL-c and AI respectively and no significant difference ($P>0.60$) for Tot-c, TG, VLDL-c, LDL-c and RBG. It also showed a low LDL-c, low TG, low VLDL-c, low Tot-c and high HDL-c which resulted in a low AI in the male gender. The RBG was increased after the injection of the leptin hormone in all the groups. There was no sexual dimorphism in RBG. The result implies that the male gender is at a lower risk developing coronary artery disease (CAD).**

***Index Terms*— Atherogenic index, Leptin hormone, Random blood glucose, Wistar rats**

I. INTRODUCTION

Leptin is a 167-amino acid peptide with a four-helix bundle moiety (motif) similar to that of a cytokine [1], [2], it is produced predominantly in the adipose tissue but is also expressed in a variety of other tissue, including placenta, ovaries, mammary epithelium, bone marrow and lymphoid tissues [3].Leptinbinds to leptin receptors (ObRs) located throughout the central nervous system and peripheral tissues [4]. The Leptin acts on receptors in the hypothalamus where it inhibits appetite, counteracting the effects of neuropeptide Y (a potent feeding stimulant secreted by cells in the gut and in the hypothalamus), and also promote the synthesis of alpha-melanocyte stimulating hormone (∝-MSH) an appetite suppressant. The absence of leptin (or its receptor) leads to uncontrolled food intake and resulting obesity [5]. Obese zucker rats had the highest plasma leptin concentrations, and Sprague-Dawley rats had the lowest (Figure 1) [6]. The half-life of circulating leptin (approximately 25min) is constant over a range of adiposity [7]. Normal values of plasma leptin in different strains of rats.[6]

Changes in plasma insulin subsequent to the consumption of high-fat diets may alter leptin production from a dipocyctes. Leptin treatment enhances insulin sensitivity in normal rats, as indicated by increased insulin-stimulated glucose utilization in peripheral tissues [8].It also decreases plasma glucose and/or insulin concentrations of normal animals in the post-absorptive state. Evidence indicates that glucose metabolism rather than insulin itself is the main determinant

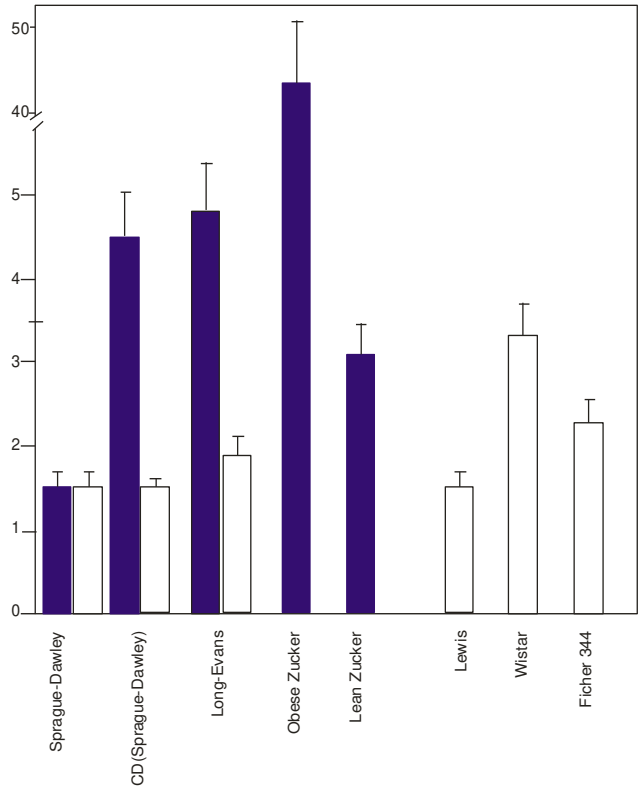

Figure 1: Plasma Leptin Concentrations in various strain of rats

for leptin expression in adipose tissue [9].

The World Health Organization (WHO) predicts 11.1 million deaths globally and 71% deaths in developing countries due to Coronary Artery Disease (CAD) by 2020 A.D [10].CAD has been associated with alterations in lipid metabolism, which include hyper-triglyceridemia and significantly reduced HDL-c [11].The increase in the concentration of cholesterol is attributable to LDL cholesterol because the high density lipoprotein (HDL) cholesterol is typically reduced [12].Leptin has been demonstrated to reduce triglycerol (TG) content in various peripheral tissues such as liver, muscle and pancreatic cells [13] and to partition free fatty acid (FFA) toward oxidation and away from storage in oxidative skeletal muscle [14]. High fat-diet in rodents result in a diminished metabolic response to peripheral leptin injections and impaired leptin transport across blood-brain barrier [14].

II. METHOD

A. Animals

Male and female Wistar rats (n = 40, male= 20, (72.9-126.4)g, female= 20, (77.2-123.0)g, age= 9 weeks), were purchased from the university of Jos, Nigeria, animal house. They were then divided into 2 main groups of male (Group A) and female (Group B) of n = 20 each. Each group was divided into 4 (four) sub-groups of n = 5 per group. The rats were studied in the course of the experiment in two different periods or phases, termed 'diet and treatment period'. The animals were housed in groups of five per cage for the period of the experiment. They were humanely treated according to the Helsinki declaration for the care of animals after obtaining clearance.

B. Grouping

Group A (male, n = 20) Group B (Female, n=20)
Group A_1 (n=5) Group B_1 (n=5)
Group A_2 (n=5) Group B_2 (n=5)
Group A_3 (n=5) Group B_3 (n=5)
Group A_4 (n=5) Group B_4 (n=5)

C. Diets

All rats were maintained *ad libitum* on the experimental diets and water for a period of 10 weeks. Four feeding regimes were used. These include:

1. Normal rat chow (group A_1 and B_1) (control diet) (100%) (standard rat chow)
2. Fat meal (group A_2 and B_2), (high-fat) margarine (90%) mixed with standard rat chow (10%).
3. Protein meal (Group A_3 and B_3), soya beans diet (100%)
4. Carbohydrate (group A_4 and B_4), cereal diet (100%)

III. TEST PROCEDURES

The Fasting serum lipid profile (f-SLP); total cholesterol (Tot-c), triglyceride (TG), very low density lipoprotein (VLDL-c), low density lipoprotein (LDL-c), high density lipoproteins (HDL-c)and RBG were measured using an automated spectrophotometer(SP-1104, P/N0-KJ2C1106, Baur Biomedical Electronics (GmbH) made in Germany, 2003) at the National Veterinary Research Institute (NVRI) Laboratory, Vom, Jos, Plateau State, Nigeria. The Atherogenic Index (AI) was calculated using Friedewaldformular:

LDL-c = Tot-c – VLDL-c
where VLDL-c = TG/2.2 [15]

Leptinhormone (Leptin rat recombinant (Cat# 213-10261, Lot# 07112118, Raybiotech.Inc, stored at 4^0C for up to one month and freeze-thaw cycles avoided) was purchased from Bio-Vision Incorporated, 155 S Milpitas Blvd. Milpitas, CA 95035, USA through Bridge Scientifik Enterprises, N0. 4 Liberty Lane G.R.A, Flower Garden, Illorin, Kwara State, Nigeria).

A dose of 3.5µg of leptin was administered intra-peritoneally daily per rat for a period of 2 weeks between 12noon – 12:30pm.

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM SPSS Inc., Chicago, IL., USA) for Windows 8.0. The results are presented in mean values ± SE. Paired t-test was used to analyze the f-SLP and RBG. One-way ANOVA was used to analyze for sexual dimorphism in f-SLP and RBG for pre-test and post- test periods. Level of significance was at $P< 0.05$.

IV. RESULTS

TABLE I: Effect of leptin hormone on f-SLP and RBG for pre-test and post-test periods

	Treatment	N	Mean	¹SD	²SEM
T-c	Pretest	37	1.79	0.24	0.04
	Posttest	36	3.48	1.01	0.17
HDL-c	Pretest	37	0.51	0.16	0.03
	Posttest	36	0.91	0.42	0.07
TG	Pretest	37	0.60	0.14	0.02
	Posttest	36	0.89	0.20	0.03
VLDL-c	Pretest	37	0.87	0.27	0.05
	Posttest	36	1.58	0.46	0.08
LDL-c	Pretest	37	0.97	0.14	0.02
	Posttest	36	1.90	0.54	0.09
AI	Pretest	37	3.84	1.10	0.18
	Posttest	36	4.47	2.07	0.35
RBG	Pretest	37	2.62	0.39	0.64
	Posttest	36	6.40	2.66	0.44

There was a significant difference (P=0.01) in the Tot-c, TG, VLDL-c, LDL-c, HDL-c and RBG in the pre-test and post-test periods, but there was no significant difference (P=0.11) for the calculated atherogenic index (AI). The results suggest that having a significant difference in f-SLP does not translate to a high or significant AI.
¹Standard Deviation, ²Standard Error of Mean.

TABLE II: Effect of leptin hormone on serum lipids and RBG for gender

		N	Mean	¹SD	²SEM
T-c	Male	36	2.57	1.13	0.19
	Female	37	2.67	1.11	0.18
	Total	73	2.62	1.12	0.13
HDL-c	Male	36	0.81	0.48	0.08
	Female	37	0.60	0.18	0.03
	Total	73	0.70	0.37	0.04
TG	Male	36	0.72	0.25	0.04
	Female	37	0.76	0.19	0.03
	Total	73	0.74	0.22	0.03
VLDL-c	Male	36	1.20	0.53	0.09
	Female	37	1.25	0.52	0.09
	Total	73	1.22	0.52	0.06
LDL-c	Male	36	1.40	0.62	0.10
	Female	37	1.45	0.60	0.10
	Total	73	1.42	0.61	0.07
AI	Male	36	3.72	1.65	0.28
	Female	37	4.57	1.60	0.26
	Total	73	4.15	1.67	0.20
RBG	Male	36	4.66	2.91	0.49
	Female	37	4.31	2.44	0.40
	Total	73	4.48	2.67	0.31

There was a significant difference (P=0.02, 0.03) in sexual dimorphism for HDL-c and AI respectively and no significant difference (P>0.60) for Tot-c, TG, VLDL-c, LDL-c and RBG. This may suggest a sexual dimorphism in HDL-c and AI.
¹Standard deviation, ²Standard Error of Mean

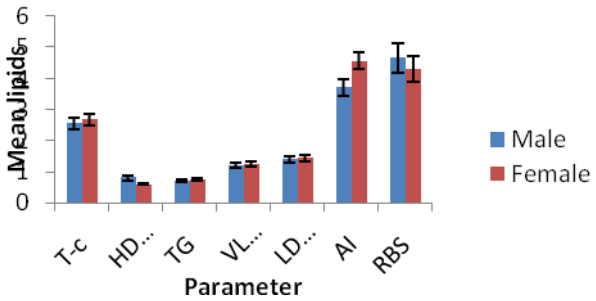

Fig.2: Comparison of the means for gender in f-SLP and RBG.

There is an obvious increase in the AI of the female subjects when compared to that of the males.

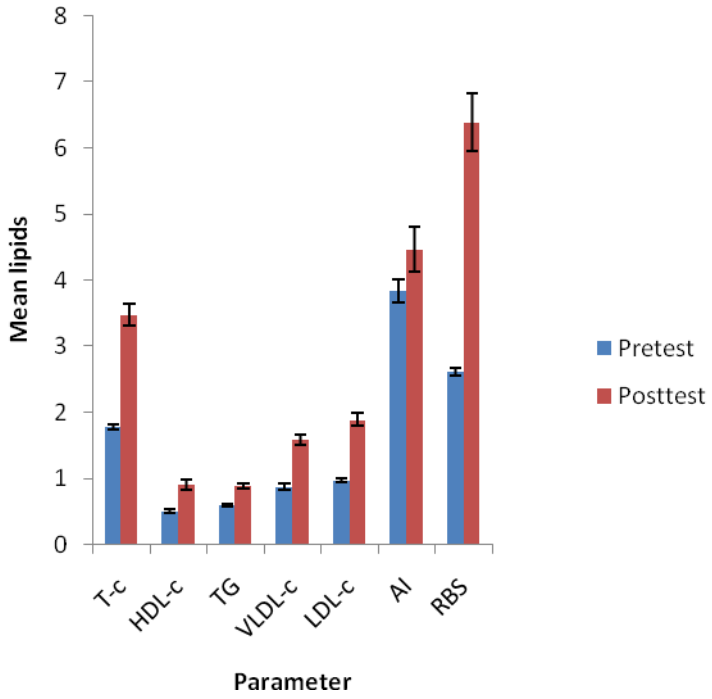

Fig.,3: Comparison of subjects for Pre-test and Post-test means in serum lipids.

There is an increase in all the measured parameters during the post-test period especially that of T-c (Tot-c) and AI.

V. DISCUSSION

A. Effect of leptin on fasting serum lipid profile and atherogenic index (AI)

Lipid profile refers to some routinely done biochemical tests to assess the atherogenic status of individuals at risk of coronary artery disease (CAD). It includes serum triacylglycerol (TG), serum total cholesterol (T-c) and its sub fractions like (High density lipoprotein) HDL-c and (low density lipoprotein) LDL-c [16]. The Framingham heart study over years has established the role of deranged lipid profile in the progression of (Coronary Artery Disease) CAD and deranged LDL-c levels are the primary target for treatment [16].

The result on f-SLP and RBG showed a significant difference (P<0.05) in the Tot-c, TG, VLDL-c, LDL-c and RBG in the pre-test and post-test periods, but there was no significant difference (P>0.05) for the calculated atherogenic index (AI), which means even though the f-SLP result is significant, the Atherogenic Index of Plasma (AIP) being insignificant showed that the animals are not at risk of developing CAD. This indicates that using only the serum lipid profile is not enough to take a decision about the significance or insignificance of a test, a further calculated lipid ratio is needed [17]. in a study on performing regression analyses, it found that AIP contributes maximum among the four ratios, approx. 31%, Castelli's Risk Index I (CRI-I) 20%,Atherogenic Coefficient (AC) with 17% and Castelli's Risk Index II (CRI-II) with 13% to the risk of developing CAD [17]. Hence, for our research AIP was used. AIP is being used by some practitioners as a significant predictor of

atherosclerosis. It has been suggested that low AIP values are associated with low cardiovascular risk and high AIP values with high cardiovascular risk [18].

A. Effect of leptin hormone on fasting serum lipid profile on sexual dimorphism

The study showed no significant difference (P>0.05) in the Tot-c, TG, VLDL-c and LDL-c and a significant difference (P<0.05) in the HDL-c and AI when the gender difference was analyzed. This result has further supported our earlier results that the leptin hormone has a more effective action on the male gender. In figure 1, the graph showed a low LDL-c, low TG, low VLDL-c, low Tot-c and high HDL-c which result in a low AI in the male. Hence the male gender is at a lower risk of developing CAD. Our study is supported by an INTERHEART- South Asia study by [19], which states that dyslipidaemia has been identified as one of the most important risk factor associated with CAD, with Low HDL-c, high TG and high LDL-c levels associated with increased incidence of CAD [19]-[20].

B. Effect of leptin on random blood glucose (RBG)

There was a significance difference (P<0.05) in the RBG between the pre-test and post-test periods. The RBG is lower in the pre-test period than the post-test period. It is higher in rats fed carbohydrate, followed by fat then protein and lower in rats fed standard rat chow. The result suggested that the administered leptin did not decrease or maintain the normal level of RBG; it increased it. One of leptin's metabolic roles is to enhance insulin sensitivity in normal rats by increasing peripheral tissue utilization of glucose [8] and hence, the glucose level normally decreases with the administration of leptin. Our research is however contrary to this but it is similar to that of [21], which stated that, there could be a different form of leptin resistance (in combination with insulin resistance and weight gain) that easily arises in laboratory animals (such as rats), as soon as they are given unlimited (*ad libitum*) access to palatable energy-dense foods [22] and it is reversed when these animals are put on low energy-density chow [23]. There was no significant difference (P>0.05) and sexual dimorphism in RBG. This may suggest that the hormone action on RBG has no predilection for gender. [24] calculated that 42% of leptin's hypoglycemic action was independent of weight reduction [24]. In contrast, the effects of leptin on whole body glucose metabolism in non-obese, non-diabetic animals remain inconsistent, but in general, short-term administration of leptin does not affect glucose metabolism [25]-[26].

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